The Relationship between Environmental Monitoring and Biological Markers in Exposure Assessment

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The poor quality of traditional assessments of exposure has encouraged epidemiologists to explore biological monitoring in studies of chronic diseases. Yet, despite theoretical advantages, biomarkers have not been widely used in such applications. This article compares the general utility of a biomarker with that of the measurement of exposure per se. Points are illustrated with a longitudinal study of boat workers in which levels of styrene in the breathing zone and in exhaled air were compared to sister chromatid exchanges (SCEs) in peripheral lymphocytes. First, the linear relationship is explored between personal exposure and the levels of a biomarker in the cohort. A good fit to the straight-line relationship reflected by a correlation coefficient which is close to 1, such as observed with styrene in exhaled air ($r^2 = 0.83$), suggests linear kinetics, that the appropriate route of exposure was measured by personal monitoring, small interindividual differences, adequate sample sizes, and a specific biomarker. However, a small correlation coefficient, as observed between SCEs and styrene exposure ($r^2 = 0.11$), indicates that either kinetics were nonlinear or that more complex issues were involved with one or more of these factors. Second, environmental and biologic measurements are compared for use as independent variables in establishing a straight-line relationship between exposure and the health effect. If the ratio of the within-person to the between-person components of variance of the independent variable is large, then significant attenuation results when estimating the slope of the line. Since such attenuation can be reduced by making repeated measurements on each person in the cohort, the sample sizes required to reduce the bias to a fixed level can be used to compare the various measures of exposure. Using data from the styrene-exposed workers, it is shown that the slope being estimated would be within 10% of the correct slope parameter with 3 personal measurements of exposure compared to 4 samples of exhaled air (12 measurements) and 20 assays of SCEs. Thus, in this case, the measurement of airborne exposure would be more efficient than that of either exhaled air or SCEs for epidemiologic purposes. — Environ Health Perspect 103(Suppl 3):49–54 (1995)

Key words: biomarkers, exposure assessment, styrene, exhaled air, SCEs, measurement error, dose–response relationship, epidemiology, variance components

Introduction

A major goal of occupational and environmental epidemiology has been to evaluate the relationships between exposures to hazardous substances and the risks of disease. Unfortunately, few exposure–response relationships have been elucidated because of the appalling lack of historic exposure data. This paucity of information about exposure has fostered applications of indirect methods to define past exposures (*1*) and has encouraged the notion that biomarkers should be used prospectively to define exposures rather than levels of contaminants in air, water, or food (*2*). In this context, the term "biomarker" refers to a measure of exposure in the form of "...an exogenous substance or its metabolite or the product of an interaction between the xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism" (*3*), and not to measures of effect or of susceptibility, which have distinct definitions (*3*).

In weighing the advantages and disadvantages of biologic and environmental monitoring to define exposure, it is clear that biomarkers enjoy at least three theoretical advantages and one major disadvantage over media-specific measurements. On the plus side, some biomarkers can substantially smooth the extreme variability in exposure typical of environmental toxicants and thereby reduce the monitoring effort (*4–6*). This is illustrated in Figure 1, which shows the exposures of six workers to inorganic lead (Figure 1*A*) and the associated levels of blood lead among the same individuals (Figure 1*B*) [data taken from Cope et al. (*7*)]. It is also true that biological monitoring accounts for all routes, i.e., inhalation, ingestion, and dermal absorption, and thereby provides a measure of the total exposure received by the individual (*3,8,9*). Finally, various biomarkers account for differences in the uptake, elimination, metabolism, and repair of toxic substances among exposed persons (*3,8,9*). However, on the minus side, the sampling and analytic demands of biomarkers are generally greater than those associated with environmental measurements and can lead to reductions in sample sizes at a given cost. Thus, it remains to be seen whether biological monitoring will supplant environmental monitoring in future investigations.

Since few studies have been published which compare levels of biomarkers with exposures in the same population, it is difficult to determine how the above strengths and weaknesses will sort themselves out. In commenting on the general utility of biomarkers in this article, points will be illustrated with data from a cohort of workers exposed to styrene in a boat-manufacturing

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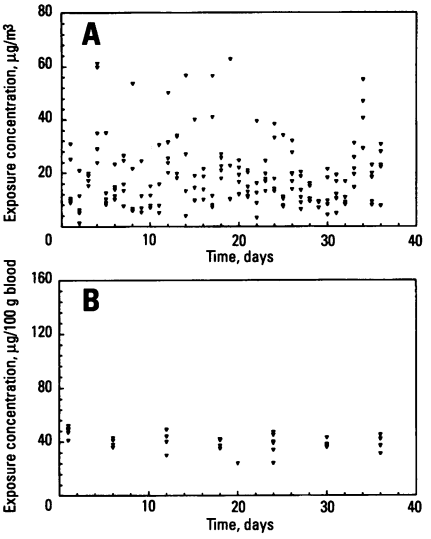

Figure 1. Exposure to inorganic lead (*A*) and levels of lead in blood (*B*) of six workers in an alkyl–lead manufacturing plant. Figures are scaled identically relative to the mean values (*7*).

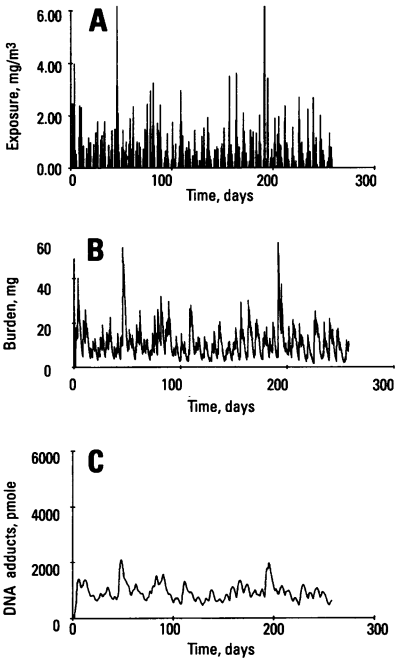

Figure 2. Theoretical relationships between (*A*) a worker's hypothetical exposure to a carcinogen for 8 hr/day and 5 days/week, (*B*) the burden of the carcinogen in the body assuming a single compartment model with an uptake rate of 1 m^3/hr and an elimination half-time of 30 hr, and (*C*) the amount of DNA adducts assuming a production rate of 1 pmole adducts/mg/hr and first-order repair with a half-time of 60 hr.

facility (*10*). The longitudinal design of that investigation allowed personal exposures and biomarkers [exhaled air and sister chromatid exchanges (SCEs)] to be measured two or more times for each individual in the cohort.

Exposure–Biomarker Relationships

To determine whether a biological or environmental indicator of exposure is likely to be superior in a particular application, it is necessary to understand the relationship between the biomarker and exposure per se. Suppose, for the moment, that only a single person is being exposed. In this context, exposure can be depicted as a series of time-varying levels of the contaminant in an appropriate medium (e.g., air). This is illustrated in Figure 2*A* for a hypothetical worker exposed to an airborne carcinogen for 8 hr/day and 5 days/week for 36 weeks. Assuming constant rates of inhalation and absorption of the substance into the body and first-order elimination from the body, the burden of the contaminant would rise and fall with the exposure series according to a pattern determined by the rate constants. This is illustrated in Figure *2B* for the case where the rate of uptake to the carcinogen is 1 m^3h^{-1} and the elimination half-time is 30 hr. Finally, under the further assumptions that the rate of production of DNA adducts is constant at 1 pmole adduct (mg carcinogen)$^{-1}h^{-1}$ and that the rate of repair of DNA is first order with a half-time of 60 hr, levels of adducts would also vary as shown in Figure 2*C*. Since the three graphs in Figure 2 are scaled identically relative to the mean values, it is clear that the time series of DNA adducts in this hypothetical person would be highly smoothed relative to that of exposure.

When kinetics are linear and the underlying exposure distribution is stationary, as illustrated in Figure 2, a strict proportionality exists between cumulative exposure (the product of the average exposure and time in Figure 2*A*), the dose of the toxic substance (the area under the curve in Figure 2*B*), and the biologically effective dose (the area under the curve in Figure 2*C*) (*5,9*). It follows from this model that the mean value of a biomarker measured repeatedly in a particular person over time (e.g., the average of point estimates from Figure 2*B* and *C*) would be proportional to his or her mean exposure over the same period; indeed, this relationship defines the implicit basis for a "biomarker of exposure" (*9*).

By extending the relationship shown in Figure 2 to a group of persons, each exposed to time-varying levels of the contaminant, an exposure–biomarker relationship between the individual mean values of exposure and the biomarker should also be linear in nature provided that the same kinetic processes and rates are shared by all persons. In this context, the fit of the linear model, as measured by the squared correlation coefficient, reflects upon the underlying linearity of the kinetic processes, the various rates of uptake, elimination, damage, and repair among the cohort, the specificity of the biomarker, and the within- and between-person components of variance in both exposure and the biomarker. Thus a good linear fit with a correlation coefficient close to one would suggest linear kinetics, appropriate route, small interindividual differences, a specific biomarker, and adequate sample size (relative to the operative components of variance). In such cases, either environmental or biological monitoring can provide valid measures of dose for epidemiologic purposes and selection of one index over another should hinge upon considerations of precision and sample size. However, a poor fit or a small correlation coefficient would indicate nonlinear kinetics or complexities associated with route, interindividual differences, specificity, and sample sizes. If the loss of correlation is associated with nonlinear kinetics, multiple routes of exposure, or interindividual differences in uptake, etc., the biomarker would be a better measure of dose. On the other hand, if the loss of correlation arises from a lack of specificity or a lack of precision of the biological assay, then environmental measurements would be preferred. Unfortunately, because it can be impossible to determine the source(s) of poor correlation between measured levels of exposure and the biomarker, the superiority of one measure over the other is often unclear in such situations and should be determined with additional studies.

Exposure to Styrene

We conducted an investigation involving the repeated measurement of personal exposures to styrene and two biomarkers among 48 boat workers (*10*). Briefly, monitoring was carried out over one year on seven occasions where the same subjects were repeatedly monitored at intervals of approximately 7 weeks. Subjects of both sexes were recruited from jobs that were expected to cover a wide range of styrene exposures. During each survey the shift-long personal exposure was monitored for each subject; and samples of mixed exhaled air were collected randomly from each subject, up to four times. In the latter case, the mean of the (typically three) measurements was used to represent the exhaled-air concentration in each subject for a given survey. Venous blood was drawn from all

subjects during four of the surveys; SCEs were measured in the lymphocytes from two of these specimens for each of 46 subjects. After accounting for absences of persons during particular surveys and for losses of specimens prior to analysis, between three and seven personal measurements of styrene exposure were obtained from each of the 48 subjects as well as three to seven samples of exhaled air (4 to 28 total measurements per subject) and one to two assays of SCEs (from only 46 subjects).

The results are presented in Figure 3, for the levels of exposure (Figure 3*A*), for styrene in exhaled air (Figure 3*B*), and for SCEs (Figure 3*C*) in each subject, as well as the associated standard errors. Because subsequent statistical analyses required normally distributed observations, the data were transformed to the natural-logarithm scale prior to analysis. Then the variance components were estimated by applying a one-way random-effects model to the log-transformed data with the ONEWAY procedure of SPSS-PC (SPSS, Inc., Chicago, IL). Figure 3 depicts the mean values and standard errors of the log-transformed data which were estimated as follows:

$$\hat{\mu}_i = \frac{1}{n_i}\sum_{j=1}^{n_i} Y_{ij} \text{ and } SE(\hat{\mu}_i) = \sqrt{\hat{\sigma}_B^2 + \frac{\hat{\sigma}_w^2}{n_i}},$$

where $\hat{\mu}_i$ represents the estimated mean (log-transformed) value for the i-th person in the group, Y_{ij} is the j-th log-transformed measurement for the i-th person (for i=1, 2, ..., k), n_i is the number of measurements obtained from the i-th person, and σ_B^2 and σ_w^2 are the estimated between-person and within-person components of variance (on the log scale) for the group as a whole. Clearly, exposure covered a wide range (0.9 – 235 mg m^{-3}; arithmetic mean = 64.2 mg m^{-3}) as was anticipated, given the various jobs represented by the workers (*10*). The corresponding ranges of the biomarkers were as follows: exhaled air ranged from 0 to 8.1 mg m^{-3} (arithmetic mean = 1.76 mg m^{-3}) and SCEs from 4.7 to 9.5 per cell (arithmetic mean = 6.4 per cell).

The regressions of ln(exhaled air) and ln(SCEs) on ln(styrene exposure) are shown in *Figure 4*. In both cases, significant linear correlation was detected (exhaled air: $p < 0.0001$; SCEs: $p = 0.011$) between styrene exposure and the respective biomarker (note that p values represent 1-tailed significance levels). However, the strength of the correlation between exhaled air and exposure (Figure 4*A*; $r^2 = 0.83$; slope = 1.11; $k = 48$) was much greater than that for SCEs and exposure (Figure 4*B*; $r^2 = 0.11$; slope = 0.028; $k = 46$). It can be concluded, from Figure 4*A* that exhaled air represents a valid biomarker of exposure to styrene in this population and that linear kinetics prevailed regarding the uptake and elimination of styrene (about 95% of the styrene dose is cleared by P450 metabolism to styrene-7,8-oxide with subsequent metabolism of this species (*11*). (Note that a strict proportionality can be demonstrated between the untransformed mean levels of the biomarker and the exposures of individuals in the sample only when the log-linear relationship has a slope of one. In the case of exhaled air, the slope of 1.11 was very close to unity, suggesting that linear kinetics prevailed regarding the uptake and elimination of styrene). The weaker relationship between SCEs and exposure to styrene, in Figure 4*B*, is more difficult to interpret. The lack of correlation could point to nonlinear kinetics or to interindividual differences among the cohort, regarding the rates of chromosomal damage and repair, or to the lack of specificity of this biomarker. The latter explanation appears more likely because it is known that cigarette smoking and other factors can increase levels of SCEs (*10*). These other factors contribute to a relatively large background value of SCEs in the general population (as well as this cohort) which tends to obscure the influence of exposure to genotoxic species. [Note: we demonstrated that styrene contributed significantly to SCEs in this cohort after controlling for cigarette consumption (*10*).]

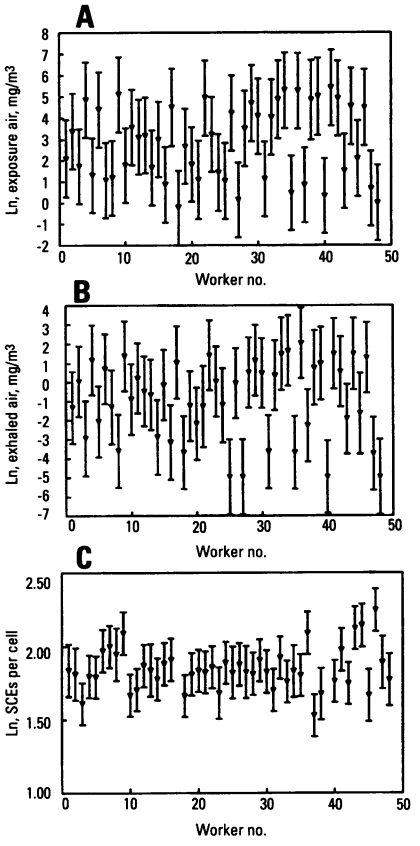

Figure 3. Exposure to styrene (*A*) among 48 boat workers measured over the full work shift (raw data given in mg/m^3), (*B*) levels of styrene in exhaled air (raw data given in mg/m^3), and (*C*) SCEs in peripheral lymphocytes (raw data given in mean SCEs per cell from scoring 80 metaphases). Estimated mean values and standard errors are shown.

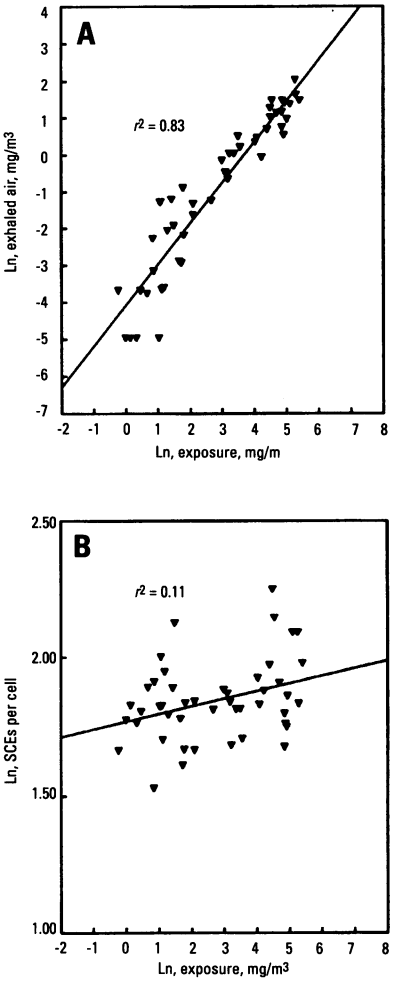

Figure 4. Exposure–biomarker relationships for boat workers exposed to styrene. Each point represents the estimated mean value of the biomarker, i.e., either (*A*) exhaled air (raw data given in mg/m^3) or (*B*) SCEs (raw data given in mean SCEs per cell from scoring 80 metaphases), plotted versus the estimated mean exposure (raw data given in mg/m^3).

Attenuation of Exposure–Response Relationships

Since exposures and levels of biomarkers are measured with error, the use of any exposure-related index as an independent variable in an epidemiological study will generally attenuate the dose-response relationship; that is, the regression coefficient will be diminished towards the null. Although such attenuation is well known in some areas of epidemiology, particularly those involving nutritional studies (*12*), it has only rarely been identified in environmental applications (*13,14*). The population components of variance, σ_B^2 and σ_w^2, can be used to assess the effect of attenuation according to the following relationship (*12–14*):

$$\beta_t = \beta_o\left(1+\frac{\lambda}{n}\right) \qquad [1]$$

where β_t = the slope in the assumed underlying straight-line relationship between true exposure (an unobservable random variable) and the continuous outcome measure, β_o=the slope which is being estimated by un-weighted least squares using the surrogate measure of the true exposure of each person (i.e., μ_i),

$$\hat{\lambda} = \frac{\hat{\sigma}_w^2}{\hat{\sigma}_B^2}$$

= the ratio of the within-person to the between-person variance components, and n = the number of measurements obtained from each subject.

In the derivation leading to Equation 1, it is assumed that both the random effect and the error term (from the one-way random-effects model) are normally distributed and that the dependent (outcome) variable is normal and free of measurement error. Other relationships are available to deal with the situation where the dependent variable is also subject to measurement error (*12*).

It follows from Equation 1 that the bias in the slope which is being estimated by the data can be expressed as a proportion of the true coefficient; that is, bias=$(1-b)$, where (*12–14*)

$$b = \frac{\beta_o}{\beta_t} = \left(1+\frac{\lambda}{n}\right)^{-1}. \qquad [2]$$

Then it becomes a simple matter to estimate sample sizes from the following expression:

$$n = \left(\frac{b}{(1-b)}\right)\lambda. \qquad [3]$$

The relationship shown in Equation 3 provides a means for comparing environmental measurements with biomarkers for applications in epidemiology at a given level of bias. What is required, of course, is information regarding the variance ratio, λ, which is rarely available. Since repeated measurements were obtained in our study of the boat workers exposed to styrene (*10*) we estimated λ as

$$\lambda\ \hat{\lambda} = \frac{\hat{\sigma}_w^2}{\hat{\sigma}_B^2}$$

by employing the estimated variance components obtained from the one-way random-effects model. In particular, the following estimates of the variance ratio were obtained: $\hat{\lambda}$ = 0.984/2.97= 0.33 for styrene exposure, $\hat{\lambda}$ = 1.35/3.16 = 0.43 for styrene in exhaled air, and $\hat{\lambda}$ = 0.022/0.010 = 2.21 for SCEs.

The variance ratios from the study of boat workers were used with Equation 3 to estimate sample sizes at levels of bias of 0.1, 0.2, and 0.4. As shown in Table 1, some interesting results emerged. It appears that personal exposure would provide the most efficient independent variable in this particular cohort for any subsequent epidemiologic investigation. In fact, if it were necessary to restrict bias to 10%, then the differences in sample sizes are striking; that is, three measurements per person would be required for airborne exposure vs. four per person for exhaled air (because each value of exhaled air in our study represented the mean of three measurements obtained for a given day, the number would actually be 3 × 4 = 12 measurements) and 20 per person for SCEs. The latter value of 20 measurements of SCEs per person is unrealistic, given the technical demands of the assay, and would probably preclude this biomarker from such an investigation. Even when the bias is as large as 40%, the need for four measurements per person makes SCEs a less likely candidate for use in such an epidemiologic study unless automated methods were available.

Table 1. Sample sizes (n) required to reduce the bias in the slope of the exposure–response relationship (which is being estimated by the data) to some proportion ($1-b$) of the slope of the true underlying relationship.

Bias ($1-b$)	Measure	n
0.1	Exposure	3
0.1	Exhaled air	4[a]
0.1	SCEs	20
0.2	Exposure	2
0.2	Exhaled air	2[a]
0.2	SCEs	9
0.4	Exposure	1
0.4	Exhaled air	1[a]
0.4	SCEs	4

(Calculations were performed with Equation 3) [a] Since one sample of exhaled air represented the mean of three measurements for each subject on a given day, the actual number of measurements would be three times the value of n.

Discussion

Although biomarkers enjoy certain theoretical advantages over environmental measurements for use in epidemiologic studies, very few relevant applications have been published. One obvious reason for this has been the difficulty of collecting and assaying biological specimens from large numbers of exposed persons. Another, but less obvious, reason probably relates to the inability to interpret results of biomarkers in the context of exposure–response relationships. If the linear correlation coefficient between the biomarker and exposure is close to one, then much can be inferred regarding the underlying processes which relate exposure with dose, and the biomarker should be suitable as an exposure-related independent variable (*5–9*). This was found to be the case for styrene in exhaled air in our study of boat workers (*10*). However, if the exposure–biomarker relationship is weak, it can be difficult or impossible to determine the reason for the lack of correlation and to decide whether the biomarker is better or worse (than exposure) as an independent variable. This was not entirely the case regarding SCEs in the styrene-exposed boat workers (because a large portion of the effect was clearly attributable to cigarette smoking), but has often been the case in studies which employed biomarkers as direct surrogates for exposure to xenobiotic substances.

Thus, we find that the application of a biomarker in an epidemiologic study to define an exposure–response relationship can be problematic. If, on the one hand, the biomarker is highly correlated with exposure, as was styrene in exhaled air, it can be used with confidence in lieu of environmental measurements. However, it may offer no

great advantage over environmental measurements in such cases, except when it is accumulated over months or years and thereby smoothes the variability of exposure within subjects, e.g., lead in blood (*4–6*). If, on the other hand, the correlation with exposure is poor, the appropriateness of the biomarker as an exposure-related variable can be unclear, particularly if data are derived from cross-sectional investigations. The lack of correlation might reflect nonlinearities or large interindividual differences in some important aspect of the exposure–dose relationship (suggesting that the biomarker is better) or a large variance ratio in either exposure (also suggesting that the biomarker is better), or the biomarker (suggesting that the biomarker is worse) or both, or might simply point to a lack of specificity of the biomarker (also suggesting that the biomarker is worse). Such a lack of specificity was responsible for the weak correlation between SCEs and styrene exposure among the boat workers since it is known that cigarette smoking induces SCEs and because it is suspected that styrene is not a strong *in vivo* inducer of SCEs (*15*).

One mechanism for dealing with the general problem of selecting among environmental and biologic measurements is to conduct a longitudinal study of a subsample of the exposed population where all monitoring options are surveyed. By repeatedly monitoring levels among the persons in the subsample, data can be used to estimate the within-person and between-person components of variance by ANOVA techniques. The estimated variance ratios, values of $\hat{\lambda}$, can then be compared among the competing exposure-related indices and sample sizes can be estimated according to Equation 3 and used in a rational manner to design the larger investigation.

Results obtained from our study of styrene-exposed workers indicated that the variance ratio was smallest for exposure ($\hat{\lambda}$= 0.33) and only slightly larger for exhaled air ($\hat{\lambda}$= 0.43). This suggests that interindividual differences in exposure and exhaled air concentrations of styrene were much larger than those operating within workers from day to day. In contrast, the variance ratio for SCEs was quite large ($\hat{\lambda}$= 2.21), due to the small interindividual differences in SCEs among the workers. Since the range of exposures was more than 200-fold, the large variance ratio of SCEs suggests that factors other than exposure to styrene were contributing to the pool of SCEs in the cohort; and, indeed, cigarette smoking was found to be a significant contributor to induction of SCEs (*10*).

Finally, it is worth repeating that the current interest in biomarkers stems in part from the general failure of occupational and environmental epidemiology to establish quantitative exposure–response relationships. However, virtually all previous investigations have been performed retrospectively in situations where little or no relevant information was available regarding exposure. Indeed, the term "exposure" can only be loosely associated with such studies since it was typically based upon either some surrogate (e.g., job title) or educated guesswork or both. The pathetic state of the historic database has, no doubt, encouraged epidemiologists to actively pursue biomarkers for use in future prospective studies. We believe that this leap-frogging of "exposure" to biologically based monitoring may be premature now that personal monitoring techniques are simple, reliable, and relatively inexpensive (at least for airborne contaminants). Certainly, our study (*10*) suggests that environmental monitoring of exposure to styrene is superior to biomonitoring in the boat-manufacturing industry. However, the results presented here should not be generalized too much since they were derived from a single investigation of one contaminant and involved just a pair of biomarkers. We encourage other investigators to conduct longitudinal studies of exposure–biomarker relationships so that the true strengths and weaknesses of the two approaches can be explored for assessing exposures to a range of xenobiotic substances.

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